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In Vitro Antibacterial Activity and β-Lactamase Stability of E-0702, a New Cephalosporin

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The in vitro activity of E-0702 was compared with the in vitro activity of cefotaxime, ceftazidime, moxalactam, and aztreonam against 600 gram-positive and gram-negative aerobic and anaerobic isolates. E-0702 had a minimal inhibitory concentration for 50% of isolates (MIC_{50}) of 25 μg for *Staphylococcus aureus*, 50 μg for *Staphylococcus epidermidis*, and 1.6 to 3.1 μg for streptococci, with *Streptococcus faecalis* resistant. E-0702 had MIC_{50}s against *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter aerogenes* comparable to those of cefotaxime, ceftazidime, moxalactam, and aztreonam, but MIC_{90}s were higher than those of the other agents. It was as active as the other agents against *Proteus mirabilis, Salmonella* spp., and *Shigella* spp., but was four- to eightfold less active against *Citrobacter freundii, Enterobacter cloacae, Providencia* spp., *Morganella* spp., and *Proteus vulgaris*, with isolates in each species resistant. Activity against *Bacteroides fragilis* was fourfold less than that of cefoxitin. E-0702 was hydrolyzed by plasmid β-lactamases and was only a weak inhibitor of plasmid and chromosomal β-lactamases. There was an inoculum effect for *E. cloacae, Serratia* spp., *Morganella* spp., and *Pseudomonas* spp.

Despite the development in the past few years of many new penicillins and cephalosporins, there has been a continued interest in novel β-lactam compounds (4). E-0702 (Fig. 1) is a cephalosporin which differs structurally from the aminothiazolylinminomethoxy cephalosporins as well as from the ureido cephalosporin cefoperazone. However, the ureido nature of the acyl side chain might provide some increased activity against certain species. If this agent is to be useful, it should be comparable or superior in activity to cefotaxime, ceftizoxime, cefoperazone, ceftazidime, or moxalactam, all of which have been shown to be clinically effective in the treatment of serious infections (5). Thus, we wished to determine the in vitro activity of E-0702 in comparison with the third-generation cephalosporins which are currently available for clinical use or are undergoing clinical evaluation. We also wished to determine its susceptibility to hydrolysis by common plasmid and chromosomal β-lactamases.

MATERIALS AND METHODS

Samples of E-0702 were a gift of Eiasi Co., Tokyo, Japan. The sources of the other compounds were as follows: cefazolin and moxalactam, Eli Lilly & Co., Indianapolis, Ind.; cefoxitin, Merck Sharp & Dohme, West Point, Pa.; cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; cefoperazone, Pfizer Inc., New York, N.Y.; ceftazidime, Glaxo Inc., Ft. Lauderdale, Fla.; and aztreonam, E. R. Squibb & Sons, Inc., New Brunswick, N.J.

Fresh dilutions of the compounds were prepared daily in either sterile medium or distilled water. Bacterial isolates were obtained from patients hospitalized at the Columbia-Presbyterian Medical Center, New York City. Many of the isolates tested were known to be multiply resistant to antibiotics and to contain β-lactamases.

Antimicrobial activity was measured by an agar dilution method with Mueller-Hinton agar unless specified otherwise. A final inoculum of 10^5 CFUs, prepared by dilution of a fresh overnight broth culture, was applied to agar by a replicating-spot device. Broth dilutions were performed with a final inoculum of 10^5 CFUs in tubes of 1 ml volume. Plates or tubes were incubated at 35°C for 18 h. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that inhibited development of visible growth on agar or in broth. The minimal bactericidal concentration (MBC) was determined by plating 0.1-ml amounts from clear 1-ml broth tubes onto blood agar plates. The MBC was defined as the concentration at which there was no growth after 24 h of incubation at 35°C. The susceptibility of streptococci was determined by using Mueller-Hinton agar supplemented with 5% sheep blood. The susceptibility of *Neisseria* and *Haemophilus* species was determined on chocolate Mueller-Hinton agar in the presence of 5% CO_2. Anaerobic susceptibility was determined by using brucella agar supplemented with sheep blood and vitamin K. Incubation of anaerobic cultures was for 48 h in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.).

TABLE 1. Comparative in vitro activity of E-0702 and other new β-lactams

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Acinetobacter spp. (25)	E-0702	≤0.01–>100	3.1	>100
	Aztreonam	0.1–>100	50	>100
	Cefotaxime	0.2–>100	12.5	>100
	Moxalactam	0.2–>100	100	>100
	Ceftazidime	0.4–>100	6.3	>100
	Cefoperazone	3.1–>100	50	>100
Aeromonas spp. (6)	E-0702	0.8–>100	3.1	3.1
Bacteroides fragilis (22)	E-0702	0.4–>100	3.1	50
	Cefoxitin	3.1–25	6.3	12.5
Bacteroides spp., other (15)	E-0702	1.6–>100	25	50
	Cefoxitin	0.8–50	3.1	12.5
Citrobacter diversus (14)	E-0702	0.4–3.1	0.8	3.1
	Aztreonam	≤0.01–0.4	0.025	0.1
	Cefotaxime	0.025–0.1	0.05	0.1
	Moxalactam	0.05–0.2	0.05	0.2
	Ceftazidime	0.1–0.4	0.1	0.2
	Cefoperazone	0.1–6.3	0.1	0.4
Citrobacter freundii (24)	E-0702	≤0.01–>100	0.4	100
	Aztreonam	≤0.01–12.5	0.1	6.3
	Cefotaxime	0.05–50	0.2	25
	Moxalactam	0.1–12.5	0.2	6.3
	Ceftazidime	0.1–>100	0.4	6.3
	Cefoperazone	0.1–>100	0.8	25
Enterobacter aerogenes (18)	E-0702	0.025–>100	0.2	12.5
	Aztreonam	0.025–25	0.1	6.3
	Cefotaxime	0.05–25	0.2	6.3
	Moxalactam	0.1–6.3	0.2	6.3
	Ceftazidime	0.2–12.5	0.4	6.3
	Cefoperazone	0.8–>100	0.4	6.3
Enterobacter agglomerans (4)	E-0702	0.025–0.4	0.05	0.4
	Aztreonam	≤0.01–>100	0.05	>100
	Cefotaxime	≤0.01–50	0.1	50
	Moxalactam	0.1–>100	0.2	>100
	Ceftazidime	0.1–6.3	0.2	6.3
	Cefoperazone	0.1–>100	0.8	>100
Enterobacter cloacae (33)	E-0702	0.05–>100	3.1	100
	Aztreonam	0.025–>100	0.1	3.1
	Cefotaxime	0.05–100	0.2	50
	Moxalactam	0.05–50	0.1	6.3
	Ceftazidime	0.05–>100	0.4	12.5
	Cefoperazone	0.1–>100	0.8	12.5
Escherichia coli (38)	E-0702	≤0.01–>100	0.1	3.1
	Aztreonam	≤0.01–0.8	0.05	0.1
	Cefotaxime	≤0.01–3.1	0.05	0.4
	Moxalactam	0.025–0.8	0.05	0.2
	Ceftazidime	0.05–6.3	0.2	0.8
	Cefoperazone	0.01–>100	0.4	25
Haemophilus influenzae (12)	E-0702	0.1–0.4	0.1	0.4
	Aztreonam	<0.1–0.2	<0.1	0.2
	Cefotaxime	<0.1	<0.1	<0.1
	Moxalactam	<0.1	<0.1	<0.1
	Ceftazidime	<0.1–0.2	<0.1	<0.1

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Klebsiella oxytoca (14)	E-0702	≤0.01–50	0.4	25
	Aztreonam	0.025–12.5	0.5	0.8
	Cefotaxime	0.025–12.5	0.5	0.1
	Moxalactam	0.1–12.5	0.1	0.4
	Ceftazidime	0.05–12.5	0.2	0.4
	Cefoperazone	0.1–>100	0.4	25
Klebsiella ozaenae (4)	E-0702	0.1–0.4	0.1	0.4
Klebsiella pneumoniae (34)	E-0702	≤0.01–100	0.2	12.5
	Aztreonam	≤0.01–0.2	0.05	0.1
	Cefotaxime	≤0.01–0.2	0.05	0.1
	Moxalactam	0.05–0.8	0.1	0.4
	Ceftazidime	0.1–1.6	0.2	0.4
	Cefoperazone	0.1–>100	0.4	25
Morganella morganii (16)	E-0702	0.4–50	6.3	25
	Aztreonam	≤0.01–0.8	0.01	0.2
	Cefotaxime	0.025–6.3	0.1	3.1
	Moxalactam	0.1–0.4	0.2	0.2
	Ceftazidime	0.05–1.6	0.1	1.6
	Cefoperazone	0.2–25	0.8	6.3
Neisseria gonorrhoeae (11)	E-0702	0.1–1.6	0.2	0.4
	Aztreonam	<0.05–0.2	<0.05	0.1
	Cefotaxime	<0.05–0.1	<0.05	0.1
	Moxalactam	<0.05–0.1	<0.05	0.1
	Ceftazidime	<0.05–0.2	<0.05	0.1
Proteus mirabilis (19)	E-0702	0.025–1.6	0.05	0.2
	Aztreonam	≤0.01	≤0.01	≤0.01
	Cefotaxime	≤0.01	≤0.01	≤0.01
	Moxalactam	≤0.01–0.2	≤0.01	≤0.01
	Ceftazidime	0.05–0.2	0.5	0.1
	Cefoperazone	0.1–>100	0.8	1.6
Proteus vulgaris (10)	E-0702	0.8–>100	1.6	>100
	Aztreonam	≤0.01–0.8	0.01	0.1
	Cefotaxime	≤0.01–50	0.05	25
	Moxalactam	0.05–12.5	0.1	0.2
	Ceftazidime	0.05–50	0.1	0.8
	Cefoperazone	0.2–50	0.8	
Providencia rettgeri (16)	E-0702	0.1–>100	0.8	>100
	Aztreonam	0.025–0.8	0.05	0.8
	Cefotaxime	0.05–1.6	0.4	1.6
	Moxalactam	0.05–0.2	0.05	0.1
	Ceftazidime	0.2–3.1	0.8	1.6
	Cefoperazone	0.2–3.1	0.4	1.6
Providencia stuartii (27)	E-0702	≤0.01–>100	0.2	25
	Aztreonam	≤0.01–>100	≤0.01	0.05
	Cefotaxime	≤0.01–0.8	0.05	0.2
	Moxalactam	0.05–0.5	0.05	0.2
	Ceftazidime	0.1–12.5	0.2	0.8
	Cefoperazone	0.2–>100	1.6	25
Pseudomonas aeruginosa (59)	E-0702	≤0.1–>100	0.2	6.3
	Aztreonam	0.2–>100	6.3	25
	Cefotaxime	0.4–>100	25	100
	Moxalactam	3.1–>100	12.5	100
	Ceftazidime	0.8–100	1.6	25

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
	Cefoperazone	0.4–>100	6.3	100
	Cefsulodin	0.4–>100	3.1	25
	Gentamicin	0.4–>100	6.3	25
Pseudomonas cepacia (19)	E-0702	0.2–>100	50	>100
	Aztreonam	1.6–>100	6.3	25
	Cefotaxime	1.6–>100	12.5	100
	Moxalactam	3.1–>100	25	>100
Pseudomonas maltophilia (13)	E-0702	3.1–>100	50	>100
	Aztreonam	6.3–>100	50	>100
	Cefotaxime	25–>100	100	>100
	Moxalactam	6.3–100	25	50
	Ceftazidime	1.6–>100	50	>100
Pseudomonas spp., other (4)	E-0702	0.05–6.3	0.8	1.6
Salmonella spp. (21)	E-0702	≤0.01–1.6	≤0.01	0.2
	Aztreonam	0.05–0.2	0.05	0.1
	Cefotaxime	≤0.01–0.8	0.05	0.2
	Moxalactam	0.1–0.4	0.1	0.2
	Ceftazidime	0.2–12.5	0.8	6.3
	Cefoperazone	0.4–>100	0.4	>100
Serratia liquefaciens (4)	E-0702	0.05–>100	0.4	>100
Serratia marcescens (34)	E-0702	0.2–>100	12.5	>100
	Aztreonam	0.05–12.5	0.1	3.1
	Cefotaxime	0.1–100	3.1	25
	Moxalactam	0.1–50	1.6	25
	Ceftazidime	0.1–12.5	0.8	3.1
	Cefoperazone	0.2–>100	1.6	>100
Shigella spp. (23)	E-0702	≤0.01–>100	0.05	1.6
	Aztreonam	0.025–0.1	0.025	0.1
	Cefotaxime	≤0.01–0.2	0.025	0.1
	Moxalactam	0.1–0.4	0.1	0.2
	Ceftazidime	0.1–6.3	0.2	1.6
	Cefoperazone	0.1–>100	0.2	3.1
Yersinia enterocolitica (6)	E-0702	0.4–1.6	0.4	0.8
Clostridium difficile (2)	E-0702	>100	>100	>100
Listeria spp. (12)	E-0702	50–>100	>100	>100
	Ampicillin	0.8–6.3	0.8	1.6
Staphylococcus aureus (12)	E-0702	25–>100	25	>100
	Methicillin	3.1–>100	6.3	25
	Cefazolin	0.2–>100	1.6	25
	Cefoperazone	0.1–>100	0.8	25
Staphylococcus epidermidis (13)	E-0702	12.5–>100	50	>100
	Methicillin	3.1–>100	6.3	>100
	Cefazolin	0.1–>100	0.8	12.5
	Cefoperazone	0.1–100	0.8	12.5
Streptococcus agalactiae (4)	E-0702	3.1–6.3	3.1	6.3
	Cefotaxime	0.1–0.4	0.1	0.2
Streptococcus bovis (4)	E-0702	1.6–3.1	1.6	3.1
	Cefotaxime	<0.1–0.	0.1	0.4

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Streptococcus faecalis (16)	E-0702	>100	>100	>100
	Ampicillin	0.8–3.1	1.6	3.1
Streptococcus pneumoniae (8)	E-0702	0.2–>100	3.1	>100
	Cefotaxime	<0.1–0.2	0.1	0.1
Streptococcus pyogenes (8)	E-0702	0.2–3.1	3.1	3.1
	Cefotaxime	<0.1–0.2	0.1	0.2
Streptococcus sanguis (8)	E-0702	6.3–>100	6.3	>100

Permeability studies were performed with mutants provided by D. Clark (1).

The presence of β-lactamase in isolates was determined by the nitrocefin assay. Stability to β-lactamase was determined by a spectrophotometric assay, using the change in absorbance at the absorption maximum of each substrate. Inhibition assays with nitrocefin were performed with a final concentration of 0.1 mM nitrocefin in a final volume of 3 ml. Enzyme and E-0702 at 0.1 and 0.01 mM were incubated at 30°C for 10 min, and then nitrocefin was added. The change in absorbance at 482 nm was followed for 10 min in a temperature-controlled recording spectrophotometer. As a control, the change in absorbance of nitrocefin plus enzyme was followed. The difference in the rate of hydrolysis during the linear part of the curve was calculated. Hydrolysis of cephaloridine was considered as a rate of 100 for comparison.

RESULTS

The overall activity of E-0702 compared with that of other agents is shown in Table 1. It inhibited half of the *Acinetobacter* isolates and had activity similar to that of ceftazidime and cefotaxime, but a significant number of isolates were resistant (MIC, >100 μg/ml). Activity against *Bacteroides* spp. was fourfold less than the activity of cefoxitin. Although isolates of *Citrobacter diversus* were inhibited at low concentrations, E-0702 was less active than the other agents, and as with *Citrobacter freundii*, a number of isolates were resistant. Some *Enterobacter* spp., particularly *E. cloacae*, were resistant. Although the MICs for 50% of isolates (MIC_{50}s) were low, they were higher than those of the other agents. *Escherichia coli, Haemophilus influenzae, Neissera gonorrhoeae, Salmonella* spp., including *S. typhi*, and *Shigella* spp. were susceptible, with MIC_{50}s of <1 μg/ml. Although E-0702 had an MIC_{50} for *Klebsiella* species comparable to those of other third-generation agents, especially cefoperazone, there were resistant isolates, and MIC_{90}s were 12.5 and 25 μg/ml. E-0702 lacked activity against most isolates of *Pseudomonas cepacia* and *Pseudomonas maltophilia*, but it was the most active agent tested against *Pseudomonas aeruginosa*, inhibiting isolates resistant to ceftazidime and aztreonam. E-0702 lacked appreciable activity against isolates of *Serratia marcescens* and was inferior to the other agents. Its activity against gram-positive species was extremely poor since *Staphylococcus aureus, Staphylococcus epidermidis*, and *Streptococcus faecalis* were resistant and the usually susceptible streptococcal species such as *S. pneumoniae, S. agalactiae*, and *S. pyogenes* required for inhibition high MICs, 3.1 μg/ml. A direct comparison of the agents against selected organisms is shown in Table 2. It is clear that other agents inhibit organisms resistant to E-0702, but E-0702 did inhibit some *P. aeruginosa* and *Acinetobacter* isolates resistant to the other agents.

Effect of growth conditions. The activity of E-0702 was determined in Mueller-Hinton agar, nutrient broth, brain heart infusion broth, and Trypticase (BBL Microbiology Systems) soy

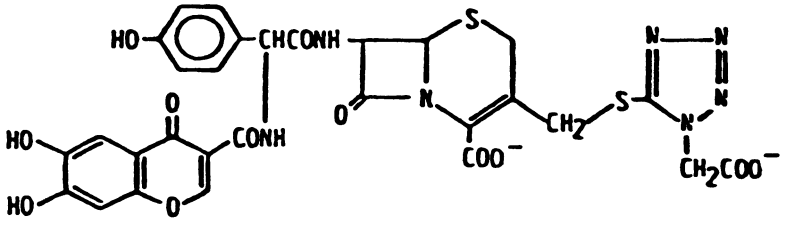

FIG. 1. Structure of E-0702.

TABLE 2. Comparison of activity of E-0702 with that of other β-lactams against selected β-lactam-resistant organisms[a]

Organism	MIC (μg/ml)				
	E-0702	Ceftazidime	Moxalactam	Cefotaxime	Aztreonam
Acinetobacter anitratus	12.5	25	>100	100	>100
Citrobacter freundii	100	1.6	3.1	6.3	1.6
Enterobacter agglomerans	0.4	6.3	>100	50	>100
Escherichia coli	1.6	3.1	0.4	0.8	0.8
Klebsiella pneumoniae	0.2	0.2	0.1	0.1	0.025
Klebsiella pneumoniae	>100	1.6	0.4	0.05	0.1
Morganella morganii	50	0.8	0.1	3.1	0.1
Providencia rettgeri	50	0.2	0.1	0.01	0.01
Pseudomonas aeruginosa	6.3	25	>100	>100	25
Serratia marcescens	>100	1.6	50	3.1	3.1

[a] All organisms were resistant to cefazolin (MIC, >32 μg/ml), carbenicillin (MIC, >256 μg/ml), piperacillin (MIC, >256 μg/ml), cefamandole (MIC, >32 μg/ml), and cefoxitin (MIC, >32 μg/ml).

TABLE 3. Effect of inoculum size upon the MICs and MBCs of E-0702

Organism	μg/ml at inoculum size (CFU) of:					
	10^7		10^5		10^3	
	MIC	MBC	MIC	MBC	MIC	MBC
Enterobacter cloacae	25	25	0.2	1.6	<0.05	0.1
Escherichia coli	1.6	1.6	≤0.05	≤0.05	≤0.05	≤0.05
Klebsiella pneumoniae	6.3	25	0.8	1.6	0.2	0.4
Morganella morganii	100	100	6.3	6.3	1.6	3.1
Proteus mirabilis	0.1	3.1	0.1	0.8	≤0.05	0.4
Pseudomonas aeruginosa	>200	>200	0.1	12.5	0.1	0.4
Serratia marcescens	200	200	0.1	1.6	<0.05	0.4

broth. There were no major differences noted for five strains each of *E. coli, E. cloacae, Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii, S. marcescens*, and *P. aeruginosa*. Similarly, no major differences were noted between MICs and MBCs within the different media. The activity of E-0702 against the aforementioned bacteria was not influenced by the pH of the medium when assays were run at pH 6, 7, and 8.

There was an effect of inoculum size (Table 3) with representative examples for each of the organisms tested. At 10^3 and 10^5 CFUs there were minimal differences between MICs and MBCs, but at 10^7 CFUs both MICs and MBCs showed marked increases for *E. cloacae, Morganella* spp., *P. aeruginosa*, and *S. marcescens*. The relation of MICs to MBCs for a large number of organisms is shown in Table 4. In most instances, the MBCs were only twofold or fourfold above the MICs. However, with *Enterobacter* spp., *Klebsiella* spp., and *P. aeruginosa*, MBCs were eightfold or greater for 40% of isolates.

TABLE 4. Relation of MIC to MBC for E-0702

Organism	No. of isolates tested	MBC/MIC ratio			
		1	2	4	≥8
Citrobacter diversus	14	2	4	5	3
Citrobacter freundii	21	8	5	3	5
Enterobacter aerogenes	18	4	4	3	7
Enterobacter cloacae	36	20	8	5	3
Escherichia coli	22	14	7	1	0
Klebsiella pneumoniae	14	6	3	2	3
Providencia stuartii	5	1	4		
Pseudomonas aeruginosa	17	4	2	3	8
Serratia marcescens	8	4	1		3

Effect of permeability. Since some of the increased activity of cefoperazone and the ureidopenicillins against *Pseudomonas* spp. seems to be related to permeability factors, we examined how E-0702 inhibited mutants of *E. coli*. E-0702 was only twofold more active against some of the mutants than it was against the parent strains. This indicates that permeability does not play a major role in the activity of this compound in terms of resistance.

β-Lactamase stability. E-0702 was not stable to hydrolysis by many of the common plasmid and chromosomal β-lactamases (Table 5). Indeed, E-0702 was less stable than cefoperazone to the TEM-1 β-lactamase and of equal stability

TABLE 5. Relative stability of E-0702 to hydrolysis by β-lactamases

Enzyme	Origin/type[a]	Relative rate of hydrolysis[b]				
		E-0702	Cefoperazone	Cefotaxime	Cefoxitin	Moxalactam
TEM-1	*Escherichia coli*/P-Pase	71	49.2	<1	0	0
TEM-2	*E. coli*/P-Pase	42	42	<1	0	0
OXA-2	*E. coli*/P-Pase	1	56.2	0	0	0
SHV-1	*E. coli*/P-Pase	61	71.1	0	0	0
PSE-1	*Pseudomonas aeruginosa*/P-Pase	82.5	12.6	10	0	0
	Morganella spp./C-Case	20.3	8.5	0	0	0
P-99	*Enterobacter* spp./C-Case	20.6	8.4	0	0	0
S-A	*P. aeruginosa*/C-Case	5	2	0	0	0
K-1	*Klebsiella* spp./C-both		2	0	0	0
	Proteus vulgaris/C-Case	34.7	8.4	4.2	0	0
	Enterobacter cloacae/C-Case (cefoxitin induced)	6	3.2	5	0	0
	Serratia spp./C-Case	19.9	14.7	<1	0	0
	Bacillus cereus/C-Case	2.9	6.7	<1	0	0

[a] P, Plasmid; C, chromosomal; Pase, penicillinase; Case, cephalosporinase.
[b] Based on a cephaloridine rate equal to 100.

to TEM-2 and SHV-1, the other common plasmid β-lactamases. E-0702, interestingly, was less stable than cefoperazone to attack by the PSE-1 β-lactamase. In terms of the chromosomal β-lactamases, E-0702 was less stable than cefoperazone, the least stable of the third-generation agents to the Richmond type 1a β-lactamases (chromosomal cephalosporinases), such as the *Enterobacter* and *Morganella* enzymes. E-0702 was also hydrolyzed by the cefoxitin-induced cephalosporinases of *Serratia* spp. It was hydrolyzed weakly by the K-1 *Klebsiella* broad-spectrum β-lactamase, which hydrolyzes aztreonam to some extent. The *Proteus vulgaris* enzyme, which hydrolyzes the aminothiazolyl-iminomethoxy cephalosporins such as cefotaxime, was less active against E-0702.

E-0702 was a relatively inefficient inhibitor of β-lactamases compared with drugs such as cefotaxime (Table 6). In no case did E-0702 function as a complete inhibitor of β-lactamase activity, as did cefotaxime.

TABLE 6. Inhibition of the hydrolysis of β-lactamases by E-0702

β-Lactamase	Relative % of hydrolysis of nitrocefin in the presence of[a]:		
	E-0702		Cefotaxime (10^{-4} M)
	10^{-5} M	10^{-4} M	
P-99	15.6	46	99.2
OXA-2	10.8	65	85.6
OXA-3	49	84	90.1
Morganella spp. (CXase)	26	51	99.6
PSE-4	7.5	41	82
Bacillus cereus	7.7	17	

[a] Based on 100% hydrolysis without E-0702.

DISCUSSION

E-0702 has a broad spectrum of antibacterial activity against most of the important members of the family *Enterobacteriaceae* and particularly against important nonfermenting bacilli such as *P. aeruginosa* and some *Acinetobacter* spp. It is most similar to cefoperazone in its in vitro activity. It has the same β-lactamase instability which cefoperazone shows with some of the plasmid β-lactamases, such as the TEM-1, TEM-2, and SHV-1 enzymes (6), but its rapid entry into bacteria seems to compensate for the defect with many bacteria, except with those which are high producers of β-lactamases. It has no permeability defects with the mutants of Clark (1) and Richmond et al. (7). It shows an inoculum effect, as does cefoperazone, for β-lactamase-producing isolates and, like all of the new agents, has an inoculum effect and differences between MICs and MBCs when tested against *P. aeruginosa*. Our results differ from those published by Katsu et al. (2), who found much lower MICs for both E-0702 and cefoperazone. This may be related to our selection of organisms which were resistant to ampicillin and to first-generation cephalosporins, such as cefazolin, as our test species.

The lack of gram-positive activity, as well as the β-lactamase instability, raises questions about the future utility of E-0702 in view of the plethora of agents which are more or equally active. In view of its structure, it is unlikely to have unusual pharmacokinetic properties in humans since it lacks a bulky acidic side chain at

position 3 of the dihydrothiazine nucleus. Nonetheless, further investigation may be of value to establish a role for this compound.

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